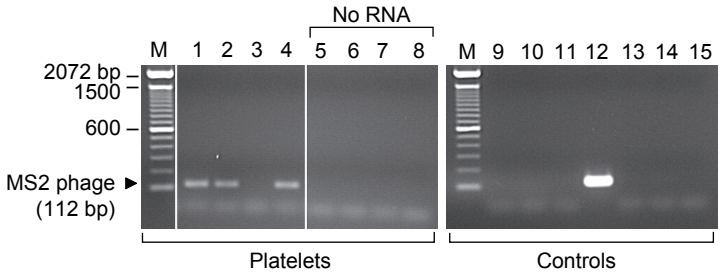
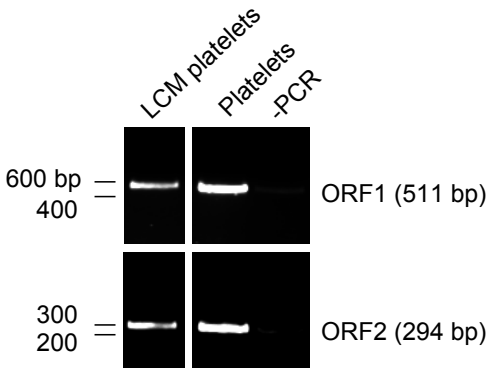
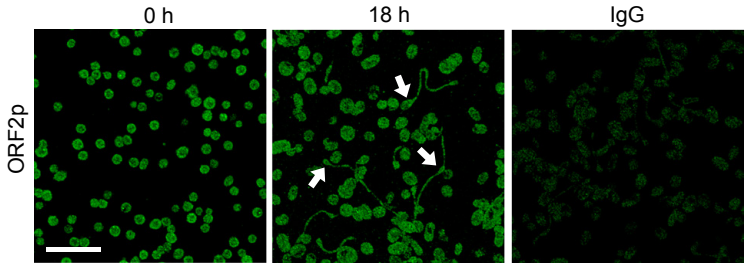


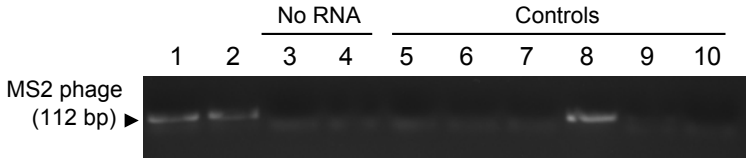
Supplemental Figure I. RT activity in human platelets is derived from a protein with a molecular weight >100-kDa. In the left panel, platelet lysates were incubated with (**lanes 1-4**) or without (**lanes 5-8**) MS2 phage RNA. The following conditions apply for the experiment: **lanes 1,5** – total platelet lysates; **lanes 2,6** – platelet lysates >100-kDa; **lanes 3,7** – platelet lysates <100-kDa; **lanes 4,8** – platelet lysates where the size exclusion fractions were recombined. The right panel displays control experiments for the study that were run in parallel. The following conditions apply: **M** - marker; **lane 9** - platelet lysate replaced with the lysis buffer only; **lane 10** - omission of the platelet lysate; **lane 11** - omission of the MS2 phage RNA; **lane 12** - platelet lysate replaced with commercial RT; **lane 13** - omission of the reverse MS2 phage primer; **lane 14** - pretreatment of MS2 phage RNA with RNase prior to the RT reaction; **lane 15** - negative PCR. This figure is representative of 2 independent experiments.



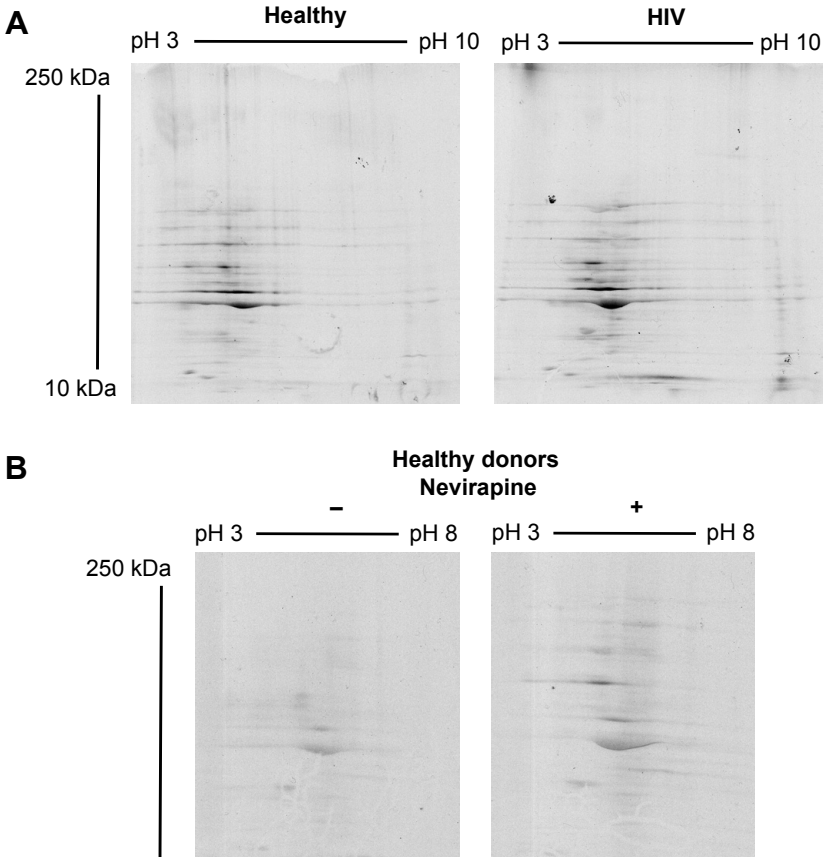
Supplemental Figure II. Single cell laser capture demonstrates that isolated human platelets express L1 *ORF1* and *ORF2* mRNA. Freshly isolated platelets were processed for immediate RNA isolation (middle lane) or fixed in suspension and spun on membrane laser microscopy (LCM) slides (left lane). Single platelets were laser captured and RNA extracted. RNA from both groups was subjected to RT-PCR to evaluate the expression of ORF1 (top panel) and ORF2 (bottom panel). This figure is representative of 2 independent experiments.



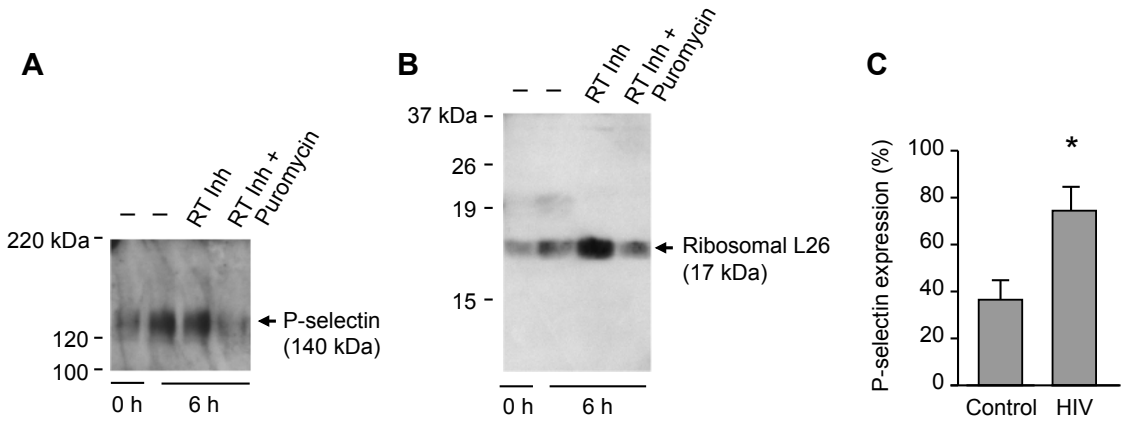
Supplemental Figure III. Human platelets express ORF2 protein. Freshly isolated platelets were fixed in suspension (0 hr) or incubated for 18 hr. The green stain identifies L1-derived ORF2 (ORF2p). The isotype specific IgG control is indicated on the right. This figure is representative of 3 independent experiments (scale bars = 20 μm).



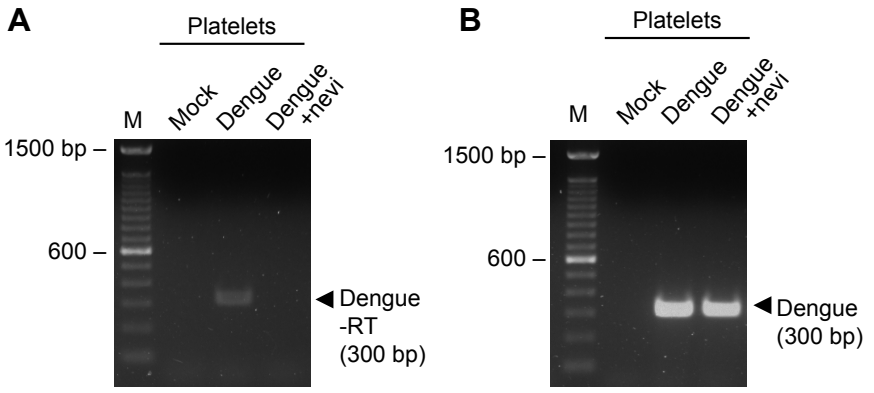
Supplemental Figure IV. Murine platelets possess endogenous RT activity. The panel displays a functional RT activity assay in highly-purified murine platelet lysates incubated with (**lanes 1-2**) or without (**lanes 3-4**) MS2 phage RNA. The following conditions apply: **lanes 1,3** – mouse platelets sample 1; **lanes 2,4** – mouse platelets sample 2. For control reactions the following conditions apply: **lane 5** – cell lysate replaced with the lysis buffer only; **lane 6** – omission of the cell lysate; **lane 7** – omission of the cell lysate and MS2 phage RNA; **lane 8** – the cell lysate replaced with commercial RT; **lane 9** – no primer; **lane 10** – negative PCR.



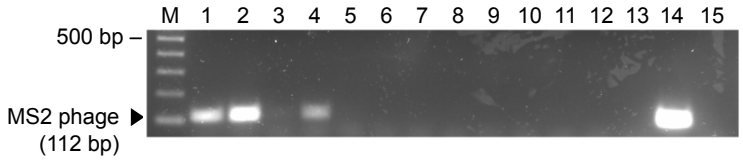
Supplemental Figure V. Endogenous RT activity increases the platelet protein expression repertoire. (A) Platelets isolated from a healthy donor (left panel) and a person with HIV (right panel) were incubated with [^{35}S]methionine and [^{35}S]cysteine to examine *de novo* protein synthesis. The two-dimensional gel autoradiography image shown in the left panel shows protein expression patterns for freshly-isolated platelets from a healthy control donor (Healthy) versus platelets isolated from a person with HIV receiving ART therapy that included an RT-inhibitor (HIV, right panel). **(B)** Platelets isolated from a healthy donor were incubated in the presence or absence of nevirapine with [^{35}S]methionine and [^{35}S]cysteine to examine *de novo* protein synthesis. The two-dimensional gels shown protein expression patterns for non-treated (NT) platelets from (left panel) versus the same platelets treated with nevirapine (right panel).



Supplemental Figure VI. Immunoblot analysis of P-selectin and RPL26 expression. Freshly isolated platelets (0h) or platelets in suspension for 6 hours (6h), treated with nevirapine (RT Inh), or RT Inh with the translational inhibitor puromycin were lysed and proteins were subsequently separated by SDS-PAGE electrophoresis. P-selectin (SELP, **A**) and ribosomal protein L26 (RPL26) (**B**) are under translational control in human platelets. (**C**) Translocation of p-selectin to the platelet surface upon stimulated with thrombin-receptor activating peptide (TRAP, 5μM, t=20 minutes) was assessed in whole blood freshly obtained from healthy controls and persons with HIV (n=5/group). Single asterisk: $p<0.05$.



Supplemental Figure VII. Endogenous RT activity in platelets modulates dengue virus replication. Platelets were infected with DENV2 (MOI 0.2) or mock virus, and treated with nevirapine (750 μM). cDNA synthesis was performed, including –RT conditions. DENV2 RNA was amplified using gene specific primer. The left gel demonstrates product amplification for the –RT reaction (i.e. detection of DENV2 DNA, which is not part of a normal virus replication cycle). The right gel demonstrates amplification of the cDNA.



Supplemental Figure VIII. Primary human cells differentially possess endogenous RT activity. The panel displays a functional RT activity assay in cell lysates incubated with (**lanes 1-5**) or without (**lanes 6-10**) MS2 phage RNA. The following conditions apply: M – marker; **lanes 1,6** – platelets; **lanes 2,7** – megakaryocytes; **lanes 3,8** – monocytes; **lanes 4,9** – T-lymphocytes; **lanes 5,10** – B-lymphocytes. For control reactions the following conditions apply: M – marker; **lane 11** – cell lysate replaced with the lysis buffer only; **lane 12** – omission of the cell lysate; **lane 13** – omission of the cell lysate and MS2 phage RNA; **lane 14** – the cell lysate replaced with commercial RT; **lane 15** – negative PCR. DNA hybrid IP. Included are gene number (ENS), location on chromosome (chr), quantification of enrichment, gene name, and the full name of the target RNAs.

Supplemental Table SI. Hybrid Target List

Gene Number	Chr	Fold Change IP vs IgG	Gene Name	Full Name
ENSG00000124657	chr6	4.68	OR2B6	Olfactory Receptor 2B6
ENSG00000205707	chr12	3.90	LYRM5	Electron Transfer Flavoprotein Regulatory Factor 1
ENSG00000243244	chr2	2.61	STON1	Stonin 1
ENSG00000140941	chr16	2.60	MAP1LC3B	Microtubule Associated Protein 1 Light Chain 3 Beta
ENSG00000182287	chrX	2.56	AP1S2	Adaptor Related Protein Complex 1 Sigma 2 Subunit
ENSG00000185305	chr5	2.52	ARL15	ADP Ribosylation Factor Like GTPase 15
ENSG00000169313	chr3	2.49	P2RY12	Purinergic Receptor P2Y12
ENSG00000150681	chr1	2.36	RGS18	Regulator of G Protein Signaling 18
ENSG00000122862	chr10	2.34	SRGN	Serglycin
ENSG00000271043	chr5	2.33	MTRNR2L2	MT-RNR2-Like 2
ENSG00000174175	chr1	1.36	SELP	P Selectin
ENSG00000161970	chr17	1.31	RPL26	Ribosomal Protein L26

Supplemental Table SI. Hybrid target list. Table includes sequenced targets being identified after anti-RNA-DNA hybrid IP. Included are gene number (ENS), location on chromosome (chr), quantification of enrichment, gene name, and the full name of the target RNAs.